Removal of Hexavalent Chromium using Chitosan prepared from the Shrimp and Crab shells

Thesis Submitted

by

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In the partial fulfillment of the requirements for the degree of

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Under the guidance of

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CERTIFICATE

This is here to certify that this thesis entitled **"Removal of Hexavalent Chromium using Chitosan prepared from the Shrimp and Crab shells"** submitted by **SAUBHAGYA RANJAN MOHANTY** (Roll No. **213CH1121)** for the award of the degree of **Master of Technology** in **Chemical Engineering**, is a bona fide record of research work which is carried out by him under my proper guidance and supervision.

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Date:

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ABSTRACT

Heavy metals are widely used in different industries. However, in this project work, the main concern is the preparation of chitin and chitosan from the raw materials of shrimp and crab shells. Then the prepared material has to be characterised by different characterisation methods. After characterisation the adsorption study has to be carried out for the chitosan prepared. The effects of parameters such as the effect of adsorbent dose, effect of contact time has to be studied in batch studies on the bio-sorption capacity. Chitin can be prepared from the waste by products i.e. shrimp shells and crab shells which involves three main stages as preconditioning, demineralization, deproteinization. Chitosan is prepared from chitin by the process of deacetylation. Then the prepared chitosan is characterised by FTIR, FESEM. With the help of batch studies, the extent of removal of the heavy metal can be compared for the two types of samples of chitosan which are prepared from the raw materials of shrimp and crab shells.

Key words: Chitin, Chitosan, Adsorption, Deproteinization, Deacetylation

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CHAPTER-1

INTRODUCTION

1. INTRODUCTION

1.1. Problem statement

Water is one of most needed and essential items for the growth and survival of every living organism. It also maintains an ecological balance between various groups of organisms and their environment [1]. A large volume of contaminated water has resulted due to the organic and inorganic wastes which are produced by various human activities which intimidate human health and other organisms.

Heavy metals are widely used in the industries like textiles, leather, paper, plastics, electroplating, cement, metal processing, wood preservatives, paints, pigments and steel fabricating industries [2]. Industrialized wastewater contains maximum quantity of heavy metals like, cadmium, chromium, lead, mercury, zinc, nickel and copper which can cause the water pollution at the time of its discharge into the nature. The presence of lower concentration of these heavy metals in water prevents the light and oxygen to penetrate into it. As a result the photosynthetic activities are reduced in the aquatic environments [3]. These also cause different direct and indirect toxic effects on humans like allergies, skin irritation, heart defects, tumours, cancer, jaundice and mutations [4].

A convenient treatment method has to be chosen for escaping the unwanted (heavy) metals from the wastewater and improve its quality before it is discharged into our environment. Various methods have been used [4]. These techniques are-

1. Membrane filtration
2. Oxidation
3. Adsorption
4. Coagulation/Flocculation
5. Chemical precipitation
6. Electrochemical reaction
7. Electro-dialysis

8. Reverse osmosis

9. Biological treatment

10. Ion exchange

Above methods show many limitations likely high capital or operating costs, low efficiency and for the small scale industries, it is difficult to handle the excessive sludge generated [4, 5]. But adsorption is preferred over all of the other methods because

- It is rapid and convenient.
- It is impenetrable to most of the types of the toxic contaminants.
- It also has very much low initial cost comparatively.
- It produces by-products which are non-toxic in nature.
- It is rather simple in design and also in operation of the treatment unit among all of these above methods.

1.2. Adsorption and its effectiveness

It is processes which often occur when a liquid solute or a gas adheres on the surface of a liquid or a solid (adsorbent), in making of an atomic or molecular film. Adsorption process is universally used in various industrial applications as in water purification and synthetic resins. There are generally two types of adsorption process:-

1. Physisorption
2. Chemisorption

1.2.1. Physisorption (or Physical adsorption):- The kind of adsorption in which the adherence of the adsorbate to the apparent is only by means of a weak intermolecular force (called van der Waals force), which is also responsible for the non-ideal behaviour of real gases.

1.2.2 Chemisorption (or Chemical adsorption):- The type of adsorption in which the adherence of the adsorbate to the surface is only by means of a chemical bond. Hence comparing the extent of adherence of the adsorbate to the surface, it is more in case chemisorption than in the case of physisorption.

Adsorption effectiveness is affected by environment and type of adsorbent like organic and inorganic materials. Ideal adsorbent should possess the following properties:-

- Bulky surface area and great adsorption ability
- Suitable pore dimension and volume
- Easily accessible
- Cost effective, not require high processing procedures
- Mechanically stable and compatible
- Easily regenerative
- Highly selective, environmental friendly

1.3. Chitin and chitosan

It is the natural amino-polysaccharides which have specific structures and multidimensional properties, [3] high range of functionality and also a wide range of applications in the biomedical and many other industrial fields.

Chitin is a long-chain homopolymer of the residues of N-acetyl-d-glucosamine which are linked to each other by osidic-1, 4 bonds [6] with the molecular formula of $(C_8H_{13}O_5N)_n$. It is a derivative of glucose, the second utmost abundant biopolymer next to cellulose, and its end product like chitosan are broadly recognized to have huge applications in several fields [4].

It is the most abundant renewable, natural resource after cellulose. Chitin and its end product are biomolecules which have excessive potential, along with flexible biological activities that demonstrates biocompatibility, biodegradability. Chitosan is a low-cost biopolymer that can be used as an ideal absorbent for removing pollutants from the wastewater. [7] It is the main module of the cell walls of fungi, the exoskeletons of crustaceans (e.g. crabs, lobsters and shrimps) and insects, the radulae of molluscs, and the beaks and internal shells of cephalopods, including squid and octopuses.

They have a wide application in the food industries, textile industries, chemical industries, medicinal fields, waste water treatment plants. [8] They are as followed:-

- health (ranging from medical sutures to beauty aids),
- water purification (coagulants for waste treatment),
- biomedical applications, agriculture (seed coatings),
- biotechnology

- nutrition (dietary supplements)
- finishing process of textile fibers

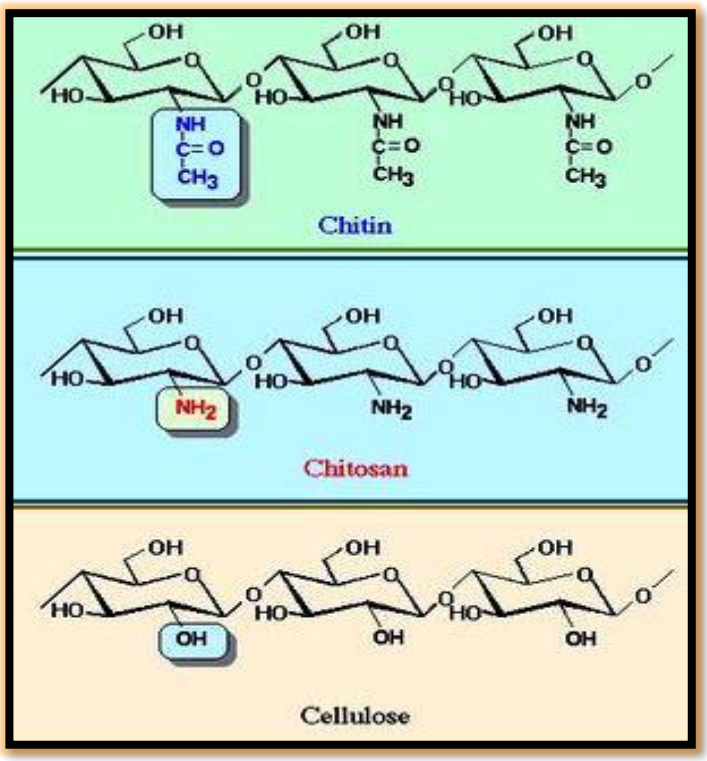

Figure 1: Structure of Cellulose, Chitin and Chitosan [9].

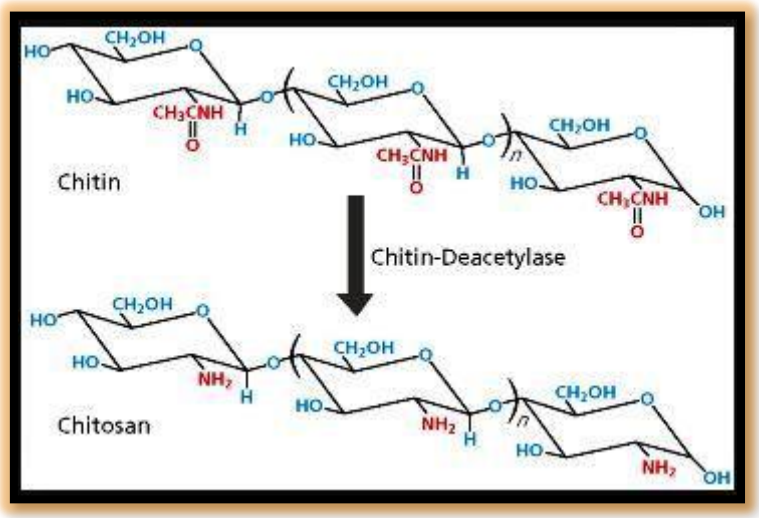

Figure 2: Chemical formula of Chitin and Chitosan [10].

1.4. Chitosan as an adsorbent

Chitosan is preferred over other various adsorbents due to its following properties which can be categorised as followed:-

- It is biocompatible [8], linear polyamine, antitumor.
- It chelates many transitional metal ions.
- Available hydroxyl groups and amino groups are reactive in nature.
- It has the effect of regeneration on the connective gum tissue.
- Both are the naturally occurring polysaccharides.
- They have the unique properties that include formation of polyxysalt, forming films, and also have optical structural features.
- Chitosan has the maximum sorption ability for metal ions among all of the adsorbents found for the heavy metals [2].
- The chitin is take out from the shells of shrimp, crab and other crustaceans. Chitosan can be achieved by the deacetylation of chitin.
- Chitosan is very much inexpensive and also abundant in nature [11].
- Chitosan chelates 5-6 more times of amounts of heavy metals in comparison to chitin because of the free amino groups which are exposed due to the deacetylation of chitin [12].

1.5. Objectives of the present work

- Preparation of Chitin and Chitosan from the raw samples of Shrimp shells and crab shells.
- Calculating the yield of chitin and chitosan from the raw samples of Shrimp shells and crab shells
- Characterization of the prepared Chitosan (FESEM, FTIR values and XRD analysis)
- Studying the effect of amount of adsorbent dose, contact time on the adsorption process.

CHAPTER-2

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1. Chitin and chitosan

If chitin and chitosan are differentiated on the basis of solubility and its nitrogen content, then chitosan is insoluble in aqueous acetic acid and soluble in lithium chloride or N, N-dimethyl acetamide solvent [i.e. LiCI/ DMAc] of 5% concentration whereas [13] the reverse is true for the case of chitin. The amount of nitrogen are less than 7% and more than 7% present in samples (purified) of chitin and chitosan respectively. From the figure of Chitosan shown below, it is concluded that in the left side extreme ring of glucopyranose ring the numbers are assigned to the six carbons, from C-1 to C-6 conventionally whereas the substitution at the carbon C-2 may be an amino group or acetamido. Generally the C-2 of the structural unit of chitosan has more than 50% of residues of acetamido (70 to 90% in common), whereas the predominance of amino groups is in chitin.

Chitin, the second most abundant polysaccharide which can be take out from the fungal species or from the exoskeletons of the sea creatures such as crayfish, lobster, prawns, crab and shrimp [14]. Chitosan is poly-(1 → 4)-2-amino-2-deoxy-b-d-glucose which is a biopolymer. It can be communicated as a biodegradable polysaccharide having higher molecular weight comparatively which is linear, heterogeneous, cationic and nontoxic in nature [15].

Chitosan can be formed from chitin by alkaline deacetylation process [16, 17]. During deacetylation process, hydrolysis of the acetyl groups of chitin occur along with the conversion to free amine groups.

The ratio between the de-acetylated units to acetylated ones is called as degree of deacetylation which can be determined with the help of this step. It is affected by various factors like time, temperature and the concentration of sodium hydroxide used in the de-acetylation [18]. This also influences the adsorption extent of chitosan. In general, higher value of DD results when comparatively higher amounts of amino groups are present that can increase the capacity of the dye adsorption of chitosan by protonation [19].

The limitations of the chitosan can be overcome by the modification of a product with the desirable properties. Its modification process is much easier in comparision to other polysaccharides as the functional groups present i.e. amino and hydroxyl groups are reactive in nature [17].

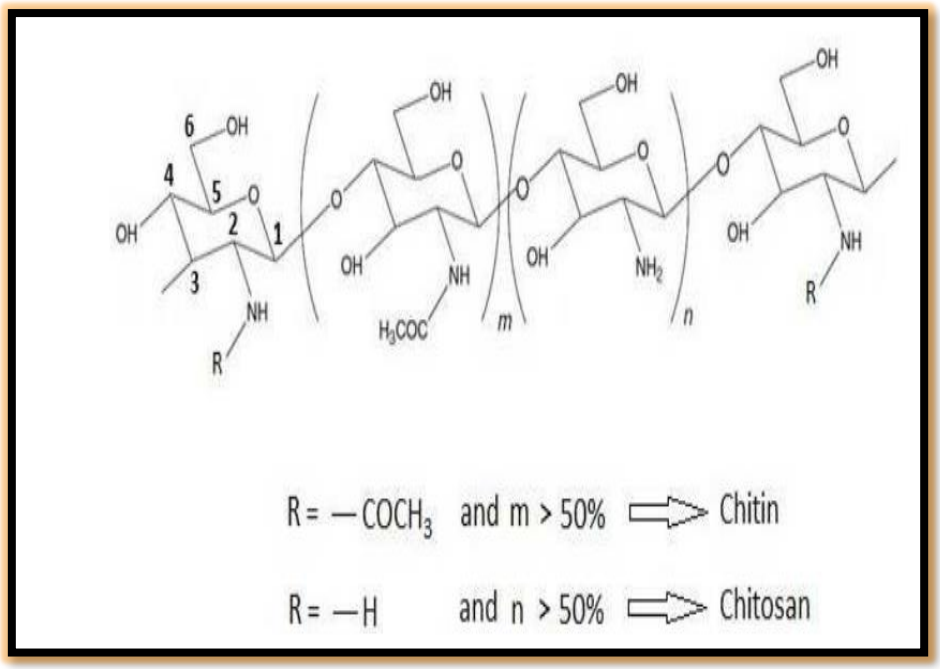

Figure 3: Comparison between Chitin and Chitosan.

2.2. Chitosan as an adsorbent

It has been studied on cost of sorbents and identify the minimum cost sorbents with prospective for treatment of waste streams and heavy metal polluted water [3]. The investigation revealed 12 potential sorbents for lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn), and mercury (Hg) [12]. Chitosan has the uppermost sorption capability for metal ions [20] identified by the investigation.

Chitosan is achieved by deacetylation of chitin, which is taken out from shrimp, crab, some fungi, and other crustaceans. Chitosan is not only economical and abundant in nature, but also is a decent adsorbent for heavy metals. Chitosan chelates 5-6 times greater amount of metals than chitin. This is assigned to the free amino groups exposed in chitosan because of deacetylation of chitin [12]. Many investigators have been studied on to amend chitosan to enable mass transfer and to depiction the active binding sites to improve the adsorption capacity. Grafting specific functional groups onto native chitosan backbone agrees its sorption properties to be improved [21].

Volesky and Holan [1] and Wase and Forster [22] studied on several bio sorbents and their capacity of metal binding including that for radioactive classes such as thorium and uranium. It has also been familiar that these bio sorbents requirement further amendment and enlargement for commercialization. Biosorbents are soft and have a propensity in aqueous solutions to agglomerate or to form a gel. In addition, the active binding sites are not readily available for sorption in their natural form. In process design transport of the metal contaminants to binding sites plays a very important role. It was also necessary to provide physical support and increase the accessibility of the metal binding sites for process applications.

Langmuir and Freundlich isotherm constants had been calculated for adsorption of Cu (II) by Findon [23] and McKay [24]. But in the literature results, any quantitative results could not be found for Cr (VI). For Cu (II) the adsorption results have been described with the Langmuir isotherm. However, Freundlich isotherm better describes the adsorption of Cu (II) which means that the adsorption sites (i.e. of heterogeneous types) in commercial chitosan are in more number. It is much difficult to make a comparision of the Freundlich adsorption constants with the Langmuir adsorption constants, but the maximum adsorption capacity (C_{max}) can be comparable with the respective literature values, particularly for non-cross-linked chitosan. Comparing the adsorption rate constant for Cr (VI) and Cu (II), the latter is likely to have greater value than the former, as smaller value of '*b'* compared to the literature.

It can be concluded from the literatures that various trace metals (Cu(II), U(VI), Cr(III), Pb(II), Cr(VI), Ni(II), Co(II), V(V) and V(IV),Fe(II), Mn (II), Pt(IV), Cd(II), Zn(II), Ir(III), Pd(II)) can be removed from the wastewater by using chitosan. In all of these studies different varieties of forms of chitosan has been used like chitosan beads, flakes and membranes shown in Table .1.

Table 1: Studies of the adsorption of metal ions using chitosan adsorbent.

Sample	Heavy metal ions	Applications	References
Chitosan/Sulfydryl-functionalized graphene oxide composites	Cu (II), Pb (II) and Cd (II)	Heavy metal removal	[25]
Chitosan grafted with with3,4dimethoxybenzaldehyde	Cd(II)	Heavy metal removal	[26]
Xanthate-modified magnetic cross-linked chitosan	Co(II)	Heavy metal removal	[27]
Ethylene-1,2-diamine-6-deoxy-chitosan	Cu(II), Pb(II), Zn(II)	Heavy metal removal	[28]
Ethylene-1,2-diamine-6-deoxy-N-phthaloylchitosan	Cu(II), Pb(II), Zn(II)	Heavy metal removal	[29]
Magnetically modified graphene oxide-chitosan composite	Cr(VI)	Heavy metal removal	[30]
Magnetic cross-linked chitosan grafted with tetraethylenepentamine	UO_2(II)	Heavy metal removal	[31]
Chitosan grafted with n-butylacrylate	Cr(VI)	Heavy metal removal	[32]
Xanthate carboxymethyl grafted chitosan	Cu(II), Ni(II)	Heavy metal removal	[33]
Montmorillonite modified with chitosan	Co(II)	Heavy metal removal	[34]
Triethylene-tetramine modified magnetic chitosan	Th(IV)	Heavy metal removal	[35]

CHAPTER-3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Raw materials

- Fresh samples of shrimp shells, crab shells can be obtained from the market.
- Both the samples were washed thoroughly with water and then dried for 24 hours.
- Then the samples are grinded with the help of a grinder separately and kept in a desiccator to avoid the moisture contact.

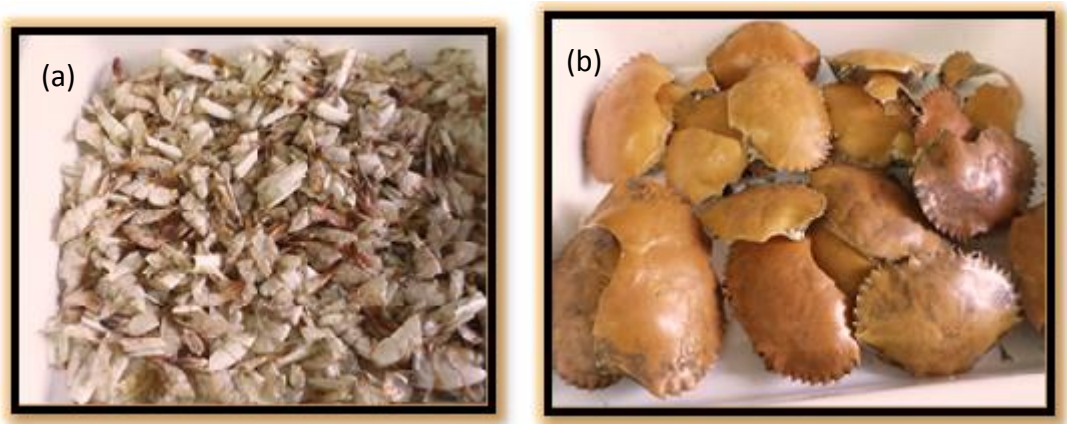

Figure 4: (a) Raw shrimp shells (b) Raw crab shells.

3.2. Chemicals and Glass wares

1. Hydrochloric Acid (HCl)- Preparing 1%,2% solution
2. Sodium Hydroxide (NaOH) pellets- Preparing 1%,2%,40% solution
3. Acetone
4. Distilled water
5. Heavy metal crystals (Cr^{+6})
6. 0.1 mol/dm^3 NaOH
7. 0.1 mol/dm^3 HCl
8. Ethanol
9. Round bottom flask
10. Condenser
11. Measuring cylinder
12. Petridis
13. Burettes and pipettes
14. Beaker
15. Filter cone

16. Filter paper
17. Scrapper
18. pH strips
19. Trays
20. Discharge pipes
21. Desiccators
22. Crucibles

All the reagents used in this work are of the analytical grade and all the solutions were prepared using distilled water. Glasswares (volumetric flasks, round bottom flask, pipette, burette weighing cylinder etc.) that are used for the experiments are all made up of Borosil. All the glasswares were thoroughly rinsed with the tap water many times and then with distilled water and then were dried in a hot air oven to remove out trace amounts of moisture present in it.

3.3. Instruments used

Table 2: List of the instruments used in this project work

Instrument	Manufacture	Function	Operation conditions
Analytical balance	Sansui (BS223S)	Weight measurement	100mg - 20g
pH meter	Systronics (361)	Measurement of pH	pH 1 to 12
Incubator shaker	Environmental orbital Shaker	Shaking of conical flasks containing samples	Speed: 100 rpm.
			Temperature: 25°C
Field Emission Scanning Electron Microscopy	FEI Nova Nano SEM 230 FESEM	To study the surface structure and chemical composition	Magnification: up to 10000X
			Resolution : 1μm
UV-spectrophotometer	Labindia	To determine the absorbance	Wavelength of 370nm
Fourier Transform Infrared	Perkin-Elmer	To predict the organic compounds present in the	Resolution of 400 cm^{-1}

Spectroscopy (FTIR)		samples	Range 400-4000 cm^{-1}
			Temp-500°C
Hot Air Oven	WEIBER	For drying of samples	Done at 70°C,105°C
Muffle Furnace	WEIBER, ADCO	For proximate analysis	As per standards
Electro-magnetic stirrer		For stirring and heating at a time	As per standards
Vaccum pump		For washing and filtering	

3.4. Methods adapted

3.4.1 Preparation of chitin from the raw samples

Chitin can be prepared from the raw samples of shrimp and crab shells. Depending upon the concentration of reagent taken, the different types of chitin are obtained. As a whole this process involves various major steps:-

1. Preconditioning
2. De-proteinization
3. Demineralization

3.4.1.1. Preconditioning

Fresh samples of shrimp shells, crab shells can be obtained from the local market. Both the samples were washed thoroughly with the water, then dried for 24 hours in the sunlight. Then the samples are grinded with the help of a grinder separately and kept in a desiccator to avoid the moisture contact. The grinded product has to be weighed and recorded.

3.4.1.2. Deproteinisation

Deproteinization of chitin is the process in which NaOH was used along with heating at 100°C. Here two concentrations of solutions were taken using 1% NaOH and 2% NaOH for both the samples (i.e. shrimp and crab).A magnetic bead has been put inside the solution and The process is carried out on the electro-magnetic stirrer for time period of 30 minutes. The solutions are continuously stirred and heated for 30 minutes. The resulting solution then washed with distilled water several times with help of a vaccum pump up to when the neutrality of the solution is not obtained (i.e. pH≈ 7). Then it is washed with ethanol.

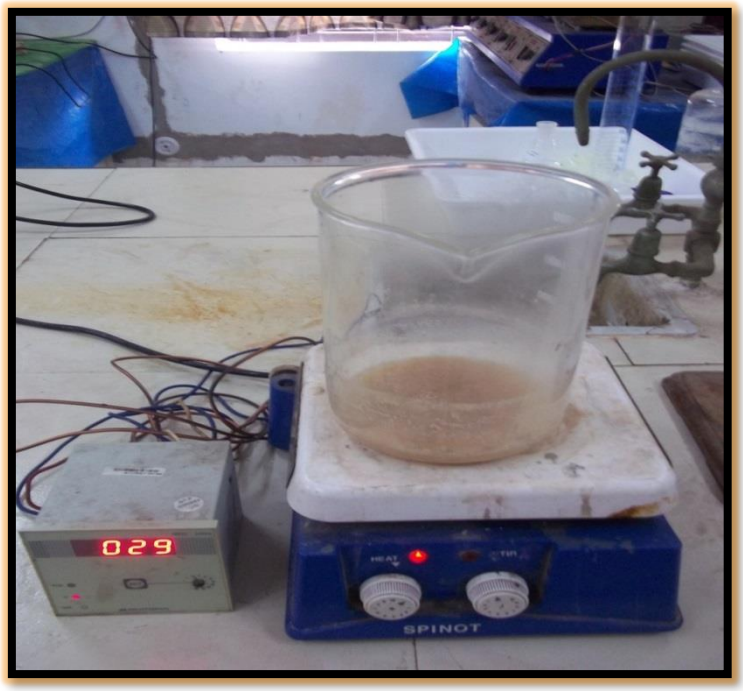

Figure 5: Deproteinisation process (over a Electromagnetic heater).

3.4.1.3. Demineralisation

Demineralization is the process in which dilute HCl solution was used without heating. Mineral content present in the shells of the crustaceans is not the same for each of the species, so all the chitin resources do not require the same type of treatments. Here the sample of shrimp shells was treated with HCl solution at ambient temperature. Here two concentrations of solutions were taken using 1% HCl and 2% HCl for both the samples (i.e. shrimp and crab). A magnetic bead has been put inside the solution and the process is carried out on the magnetic stirrer for time period of 30 minutes. The solutions are continuously stirred. The resulting solution then washed with distilled water several times with help of a vaccum pump up to when the neutrality of the solution is not obtained (i.e. pH≈ 7). Then it is washed with

ethanol .Then the demineralized sample is then filtered with the help of the vaccum pump and dried in an oven for a period of 5 hours at 60°C. Then it is weighed which is none other than purified chitin.

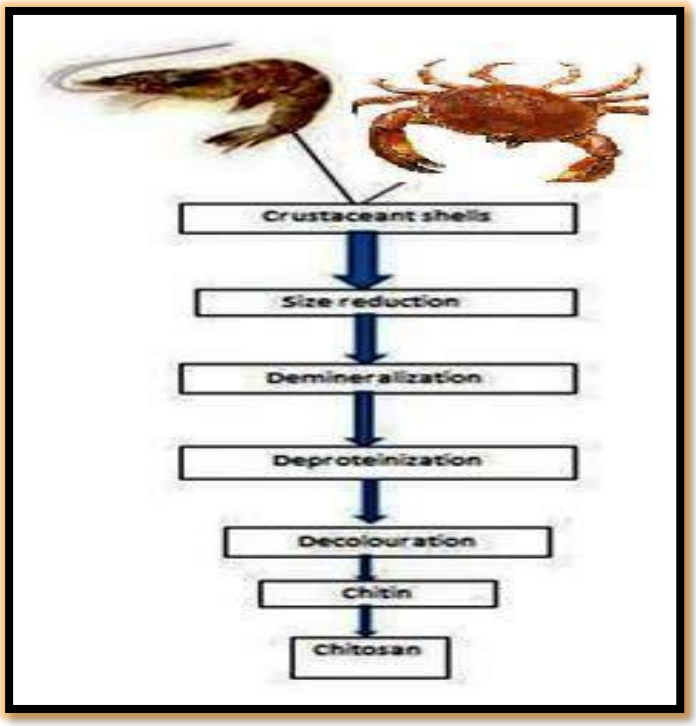

Figure 6: Flow diagram of process of preparation of Chitin.

Figure 7: Chitin samples from Crab and Shrimp raw materials.

3.4.2. Preparation of chitosan from chitin

Chitosan can be prepared from Chitin by the following method which has three steps:

1. Deacetylation
2. Purification
3. Drying

3.4.2.1. Deacetylation

Deacetylation is the process with the help of which chitosan can be prepared from chitin which has been prepared from the raw, grinded samples of shrimp and crab shells. In this process, sample is treated [36] with concentrated NaOH (40%).

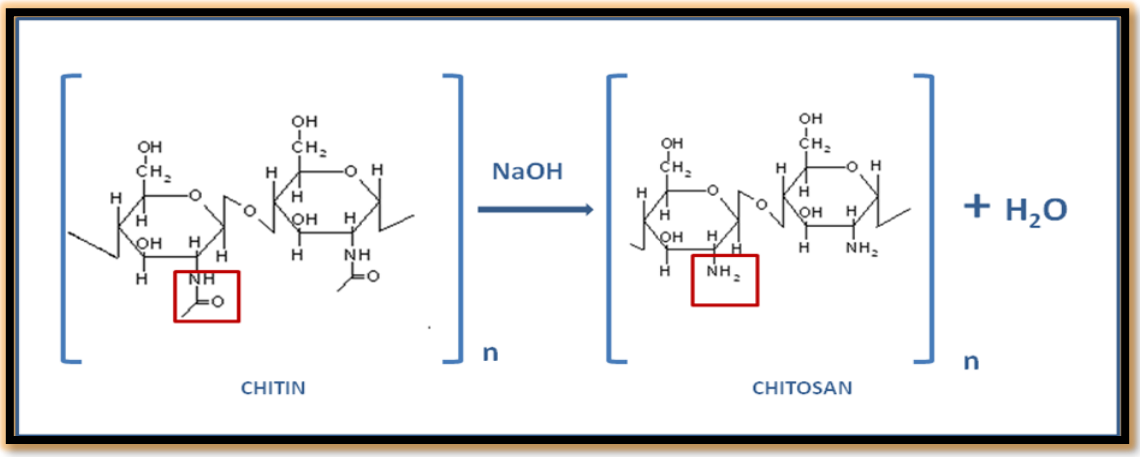

Figure 8: Deacetylation process.

The weighed sample of chitin is taken in a round bottom flask and 40% NaOH is added to it. A solution is prepared with 1/10 (w/v) ratio. Then the flask is put inside a water bath (A beaker which is half filled with water). The beaker is placed on the magnetic stirrer cum heater and a condenser is also fitted with the round bottom flask. Continuous water is supplied to the condenser from the tap through the discharge pipe for effective deacetylation process. The temperature is maintained throughout the process is 100°C. The temperature probe should be put in the water bath to check out the temperature of bath. Continuous water is added to the water bath within certain intervals of time for precautionary safety cause. The heating process is carried out for a period of five hours.

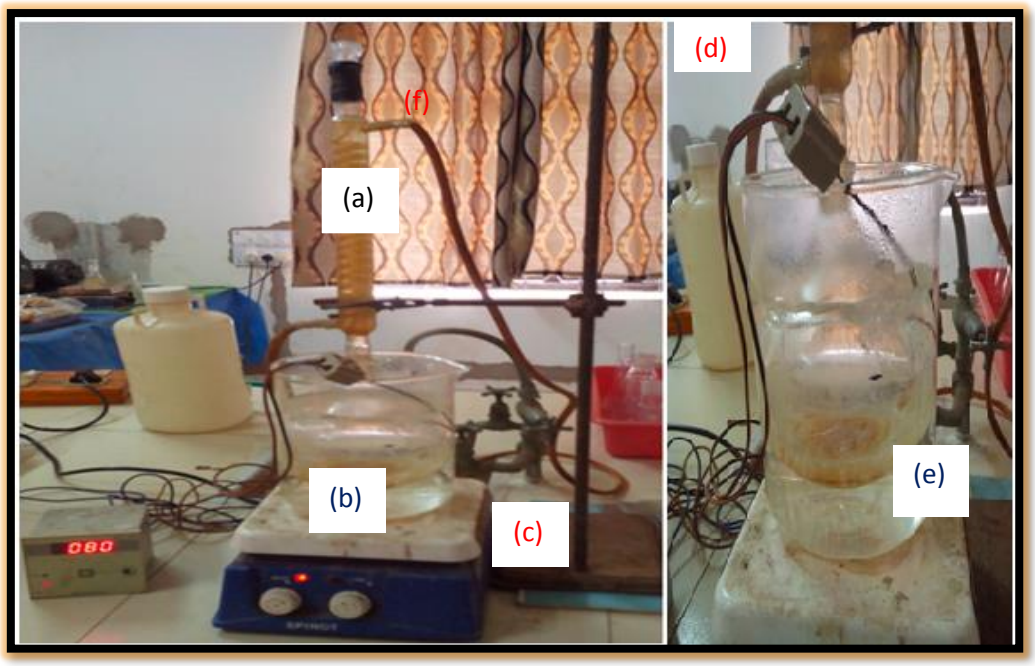

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Figure 9: Deacetylation Process and the sample inside the round bottom flask,(a)condenser (b) Water bath (c) Electromagnetic heater (d) Temperature probe (e) sample inside the round bottom flask (f) Discharge pipes.

3.4.2.2. Purification

After the deacetylation process ,the prepared sample from the flask is taken and washed several times with the help of the vaccum pump up to the time when the solution became neutral (i.e. pH≈ 7). When the neutrality of the solution is obtained, the sample is filtered properly with the filter paper. Then it is washed with ethanol.

3.4.2.3. Drying

The filtrate is then dried at 60°C in an oven for a time period of 5 hours. Then the dried samples can be collected with the help of a scrapper and weighed. The weighed sample is then grinded with the help of mortar. The sample prepared is chitosan. Depending upon the concentrations of the reagents (HCl and NaOH), 1% and 2% chitosan are prepared.

Figure 10: Chitosan samples prepared from Shrimp and Crab shells.

Summarising the whole methodology of preparation of chitosan from the the raw materials of shrimp shells and crab shells as [36] followed**:**

Raw materials -→ Size reduction-→ Preconditioning -→ Deproteinisation (dil. NaOH) →Washing -→ Demineralisation (dil. HCl) -→ Washing and dewatering -→Decolouration-→Chitin-→ Deacetylation (conc. NaOH)-→ Washing and dewatering-→ Purification and Drying-→ Chitosan

3.4.3. Characterization

3.4.3.1. Chemical analysis (Proximate Analysis)

The proximate analysis is one of the important methods of characterization of a substance by means of which the distribution of various molecules or the chemical composition can be obtained when the provided sample is heated under specified conditions. It is the most often used analysis for characterizing a material in connection with their utilization. This analysis analyses the sample into 4 groups i.e. Moisture; gases and vapours present (i.e. volatile matter) ; the non-volatile fraction (Fixed carbon) ; the inorganic residue remained after combustion (i.e. ash).

Therefore by means of the proximate analysis, we can study and analyse 4 different contents inside the material. They are:-

1. Moisture Content
2. Volatile Matter Content

3. Ash Content
4. Fixed Carbon

3.4.3.1.1. Moisture content

Moisture content is the water content in the material at the time when it is being sampled. By means of this, the amount of moisture present can be determined in the sample by calculating the loss in mass between the sample (before heating) and the sample which has been heated under controlled conditions (the water which is not contained in its chemical structure can be taken off). The sample should be heated in a hot air oven for a period of one and half an hour in a petridis at a steady temperature of $105 \pm 5°C$.

$$M = (w_2 - w_3)/ (w_2 - w_1) *100 \qquad (1)$$

Where

w_1 is weight of the empty Petridis

w_2 is weight of the empty Petridis + sample before heating

w_3 is weight of the empty Petridis + sample after heating

3.4.3.1.2. Volatile matter

Volatile matters are those which include the components of the material, which are liberated at high temperature in absence of oxygen other than water. Volatile matter determination is an essential method for safety concern as the high volatile content can lead to spontaneous combustion which is very much hazardous. The volatile matter content in the provided sample can be calculated by measuring the mass of volatiles before and after heating under controlled conditions in the muffle furnace. It can be determined by heating the sample for 7 minutes in a translucent silica crucible (of cylindrical shape) with a lid at a steady temperature $925 \pm 10℃$ in a Muffle furnace.

$$V (\%) = [(w_8 - w_9)/ (w_8 - w_7) *100] \qquad (2)$$

Where

Weight of the empty crucible (w_7)

Weight of the empty crucible + sample of shrimp shells before heating (w_8)

Weight of the empty crucible + sample of shrimp shells after heating (w_9)

3.4.3.1.3. Ash content

It is the inorganic residue content that still remains after the removal of water and organic matter when heat is applied in the presence of oxidizing agents. It also provides the total amount of minerals within any food. Ash content is obtained by heating the sample for one and half an hour in a translucent silica crucible at a steady temperature of 550 ± 10℃ in a Muffle furnace.

$$A = (w_6 - w_4)/ (w_5 - w_4) *100 \qquad (3)$$

Where

w_4 is the weight of the empty crucible

w_5 is weight of the empty crucible + sample before heating

w_6 is weight of the empty crucible + sample of shrimp shells after heating

3.4.3.1.4. Fixed carbon

It can be obtained by subtracting the percentages of moisture content, volatile matter content and ash content from the original mass of the raw sample(taken before it is treated under specified conditions), solid residue which remains after all the volatiles driven off from this particular sample.

$$F = 100 – (V + A + M) \qquad (4)$$

Where V is volatile matter, A is ash content, M is Moisture content.

3.4.3.2. Fourier transform infrared (FTIR ANALYSIS)

Infrared spectra were obtained using a Perkin-Elmer type FTIR 1000 spectrometer at room temperature and using KBr pellet scanning method. Pellets were scanned at room temperature (25°C) in the spectral range of 400 – 4000 cm-1. Fourier transform infrared (FTIR) spectroscopy was used to confirm the formation of chitin and chitosan (synthesized from forming chitin) from the fish and shrimp shells. FITR spectrometer was obtained under dry air at room temperature using KBr pellets.

3.4.3.3. Field Emission scanning electron microscopy (FESEM)

FESEM is one of the the popular analytical techniques that provide the information about the surface structure of the particular sample. In this technique, an electron gun generates electron which enter into the surface of the sample and many secondary electrons of low energy are generated. These secondary electrons have the intensity which is governed by topography of surface of this sample. The position of the scanning primary electron beam will give the measurement of the intensity of the secondary electron. From this, the image of the surface of the sample can be obtained. The preparation of the sample is done without coating as it is a powdered sample of a biopolymer material. Scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM/EDX) is the best known and most widely-used of the surface analytical techniques. High resolution images of surface topography, with excellent depth of field, are produced using a highly-focused, scanning (primary) electron beam. The primary electrons enter the surface with an energy of 0.5 – 30 kV (JEOL JSM-6084LV, ACC V 30kv, working 8mm, magnification: 8x to 300000x) and generate many low energy secondary electrons.

3.4.4. Preparation of stock solution

A stock solution of heavy metal has to be prepared for the different batch experiments conducted for the adsorption process. 100 ppm of stock solution is prepared i.e. 100 mg (0.1gm) of chromium (Cr^{+6}) crystals are poured into a volume of 1000 ml of distilled water which is taken in a beaker. Then it has to be stirred properly for a homogeneous concentration.

Figure 11: Stock solution prepared.

3.4.5. Batch experimental procedure

The adsorption of heavy metal (Cr^{+6}) was studied using the chitosan sample (crab and shrimp shells) in the batch operation for contact time of 60 minutes. First 30 ml of heavy metal solution was taken in the 150 ml conical flask and then a known amount of the chitosan was poured into the conical flask. Then it was put in to the shaker at 100 rpm. Each sample of liquid (1ml) was pipetted out at regular time interval of time for 60 minutes of contact time. Collected liquid sample was subjected to centrifuge till clear liquid was separated from chitosan. Using UV- Spectrophotometer at Λ_{max} 370 the absorbance of clear liquid sample was estimated. To obtain the dye concentration the calibration curve was plotted and the absorbance of the unknown dye solution obtained from spectroscopic analysis was used to estimate the dye concentration.

3.4.5.1. Effects of various parameters on adsorption of chitosan

1. Study the effect of contact time
2. Study the effect of adsorbent Dosage

3.4.5.1.1. Study the effect of contact time:

For the study of contact time on the adsorption of chitosan, 30 ml from 100 ppm Cr^{+6} solution is taken in a conical flask and 0.1gm of chitosan (both of crab and shrimp separately) is added in the flask at solution pH The flask was kept at 25 ^{0}C in the shaker at 100rpm shaking speed. Then the sample was pipetted out at the interval of 60 minutes initially and then at each interval of 60 minutes. Within the first hour of study, the concentration in the remaining sample was analyzed for absorbance in the UV-spectrophotometer.

3.4.5.1.2. Study the effect of adsorbent Dosage:

Effect of adsorbent Dosage on the adsorption of Cr^{+6} is studied by taking 30 ml from 100 ppm Cr^{+6} solution in a conical flask and then different amount of chitosan were added in the different conical flask at certain pH. After we keep the conical flask at 25 ^{0}C in the shaker at 100rpm, the sample was collected at a certain time interval to obtain the concentration remaining in the solution after adsorption.

CHAPTER-4

RESULTS AND DISCUSSIONS

4. RESULTS AND DISCUSSIONS

4.1. Prepared chitin and chitosan

Here two types of chitin and chitosan are prepared from the corresponding sample of raw materials of shrimp and crab shells. Both the types are also of two different concentrations namely 1% and 2% as according to the concentration of the reagents taken for the preparation.

Table 3: Yield of the sample of chitosan prepared.

Raw Materials	Conentration of the reagents taken	Initial amount of raw materials (gms)	Chitin (gms)	Chitosan (gms)	Yield (%)
Crab	1%	20	13.915	4.72	23.60
Crab	2%	30	21.450	7.65	25.50
Shrimp	1%	20	5.309	2.35	11.75
Shrimp	2%	30	11.061	6.50	21.66

4.2. Proximate analysis

4.2.1. Proximate Analysis of raw sample of Shrimp shells

4.2.1.1. Moisture Content

Weight of the empty Petridis (w_1) = 34.193gm

Weight of the empty Petridis + sample of shrimp shells before heating (w_2) = 44.193gm

Weight of the empty Petridis + sample of shrimp shells after heating (w_3) = 43.605gm

$$M = (w_2 - w_3)/ (w_2 - w_1) *100$$

$$= 0.0588 *100$$

$$= 5.88$$

4.2.1.2. Ash content

Weight of the empty crucible (w_4) = 17.644gm

Weight of the empty crucible + sample of shrimp shells before heating (w_5) = 18.644gm

Weight of the empty crucible + sample of shrimp shells after heating (w_6) = 17.913gm

$$A= (w_6 - w_4)/ (w_5 - w_4) *100$$

$$= 0.269 *100$$

$$= 26.9$$

4.2.1.3. Volatile matter

Weight of the empty crucible (w_7) = 15.730gm

Weight of the empty crucible + sample of shrimp shells before heating (w_8) = 16.730gm

Weight of the empty crucible + sample of shrimp shells after heating (w_9) = 16.031gm

Volatile Matter (%) = [(w_8 - w_9)/ (w_8 - w_7) *100] - Moisture content (%)

= [0.699 *100] – 5.88

= 69.9 – 5.88

= 64.0

4.2.1.4. Fixed carbon

F = 100 – (V + A + M)

= 100 – 5.88 – 64.02 – 26.9

= 3.2

4.2.2. Proximate analysis of raw sample of crab shells

4.2.2.1 Moisture content

Weight of the empty Petridis (W_1) = 34.146gm

Weight of the empty Petridis + sample of shrimp shells before heating (W_2) = 44.146gm

Weight of the empty Petridis + sample of shrimp shells after heating (W_3) = 43.793gm

M = (W_2 - W3)/ (W2 - W_1) *100

= 0.0353 *100

= 3.53

4.2.2.2. Ash content

Weight of the empty crucible (W_4) = 21.032gm

Weight of the empty crucible + sample of shrimp shells before heating (W_5) = 22.032gm

Weight of the empty crucible + sample of shrimp shells after heating (W_6) = 21.483gm

A = (W_6 – W_4)/ (W5 - W_4) *100

= 0.451 *100

= 45.1

4.2.2.3. Volatile matter

Weight of the empty Petridis (W_7) = 14.482gm

Weight of the empty Petridis + sample of shrimp shells before heating (W_8) = 15.482gm

Weight of the empty Petridis + sample of shrimp shells after heating (W_9) = 14.937gm

$$V = [(W_8 - W_9)/ (W_8 - W_7) *100] - M$$

$$= [0.545 *100] – 3.53$$

$$= 54.5 – 3.53$$

$$= 50.97$$

4.2.2.4. Fixed carbon

$$F = 100 – (V + A + M)$$

$$= 100 – 3.53 – 50.97 – 45.1$$

$$= 0.4$$

Table 4: Proximate Analysis of the sample of Shrimp shells and crab shells.

Samples	Moisture content (%)	Ash content (%)	Volatile Matter (%)	Fixed Carbon (%)
Shrimp shells	5.88	26.9	64.02	3.2
Crab shells	3.53	45.1	50.97	0.4

4.3. FTIR analysis:

Preapared chitosan from crab shells using different concentrations (1% HCl & 1% NaOH and 2% HCl & 2% NaOH) of HCl and and NaOH are shown in the figures (12& 14). In the FTIR spectra of crab chitin (1% HCl & 1% NaOH) , we found some peaks in th region of 3793 cm^{-1}, 3250cm^{-1}, 2979cm^{-1}, 2336 cm^{-1}, 1643 cm^{-1}, 1392 cm^{-1} , 1020 cm^{-1}, 860 cm^{-1}, 579 cm^{-1} . The ftir spectra of Crab chitn (2% HCl & 2% NaOH) is around 1420 cm^{-1} and 836 cm^{-1} . It is clear that these spectra peaks around 1020 cm^{-1} and 860 cm^{-1} because of some hyroxypaptite mineral is there and also proteins componds, So it is clear that these concentrations of HCl and NaOH are not sufficient for removal of protiens and minerals . So it is clear that increase the concentrations in the demineralization process. Some modifications are necessary for removal of minerals [36].

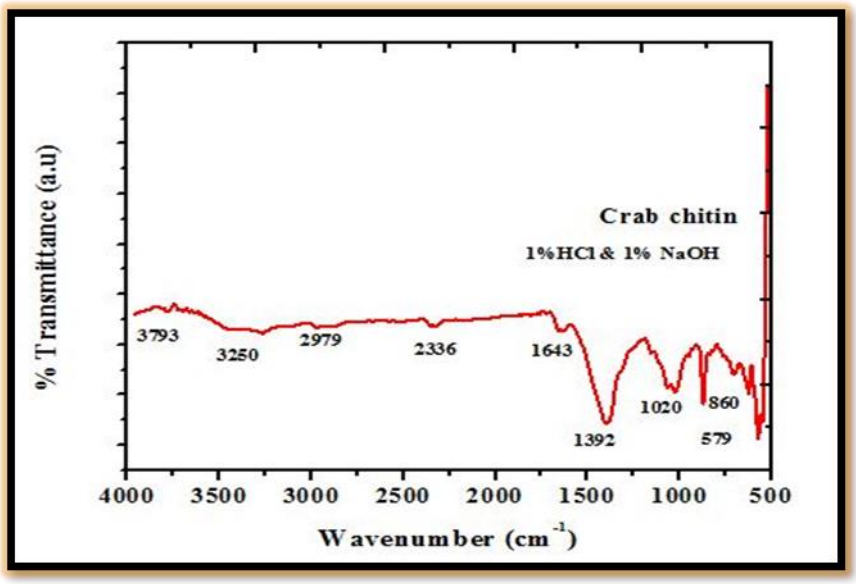

Figure 12: Crab chitin (1% HCl and 1% NaOH).

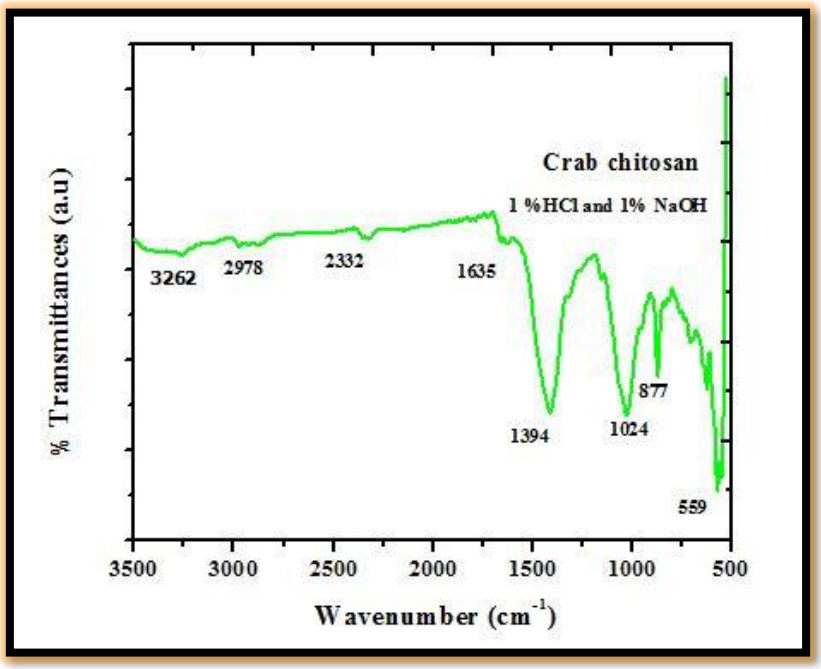

Figure 13: Crab chitosan (1% HCl and 1% NaOH).

The FTIR spectra of shrimp chitin (1% HCl & 1% NaOH) , we found some peaks in th region of 3460 cm-1, 3260 cm-1, 3107cm-1, 2926 cm-1, 2857 cm-1, 1626cm-1 , 1557 cm-1, 1368 cm-1, 1015 cm-1 , 851cm-1and 625cm-1 . The ftir spectra of shrimp chitn (2% HCl &

2% NaOH) are around 1420 cm-1 and 836 cm-1.Similarly results found that in the case of shrimp chitin in the different concentration of HCl and NaOH.

The FTIR spectra of shrimp chitosan (1% HCl & 1% NaOH,), we found some peaks in the region of 3443 cm-1, 3262 cm-1, 3107 cm-1, 2874 cm-1, 1626 cm-1, 1557 cm-1 , 1368 cm-1, 1299 cm-1, 1075 cm-1 , 1015 cm-1, 903 cm-1 and 541cm-1 . The FTIR spectra of shrimp chitosan (2% HCl & 2% NaOH) are around 3443 cm-1, 3262cm-1, 3107 cm-1, 2874 cm-1, 2349 cm-1, 1626cm-1, 1549cm-1,1376cm-1,1299cm-1,1075cm-1,1006cm-1,903cm-1and 628cm-1.It is clear that for FTIR spectra, some peaks like 1549cm-1regoin shown that shrimp chitin is prepared by this concentration, this is sufficient for removal of minerals in the raw shrimp shells, So it is also clear that in the both spectra, treatments depends on the raw materials compositions [37,38].

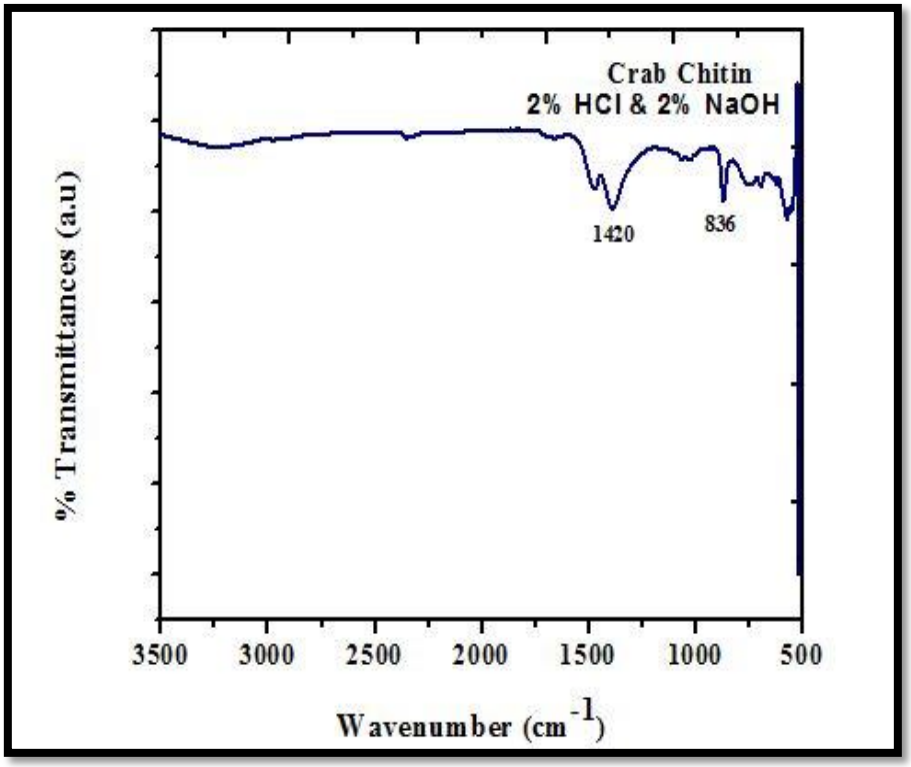

Figure 14: Crab Chitin (2% HCl and 2% NaOH).

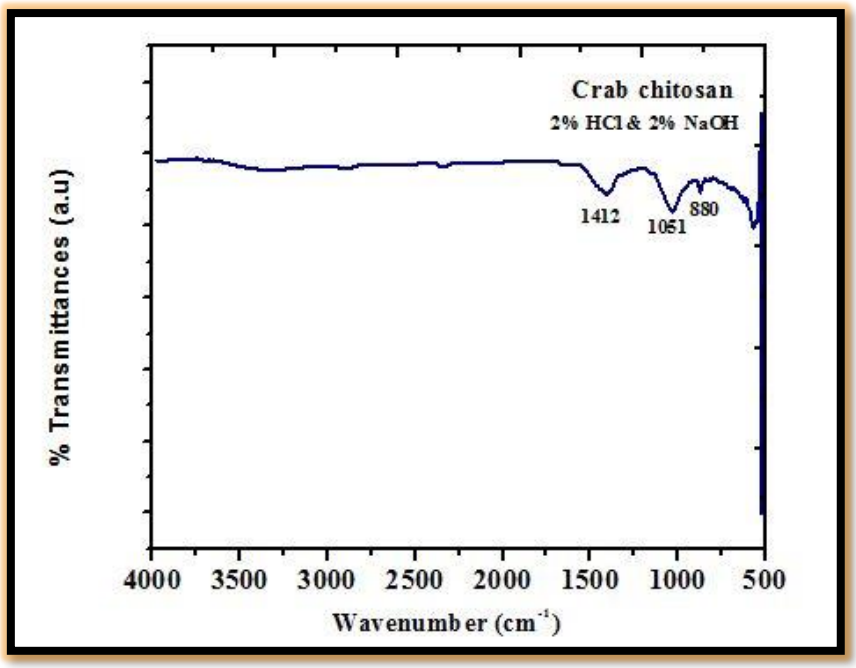

Figure 15: Crab chitosan (2% HCl & 2% NaOH).

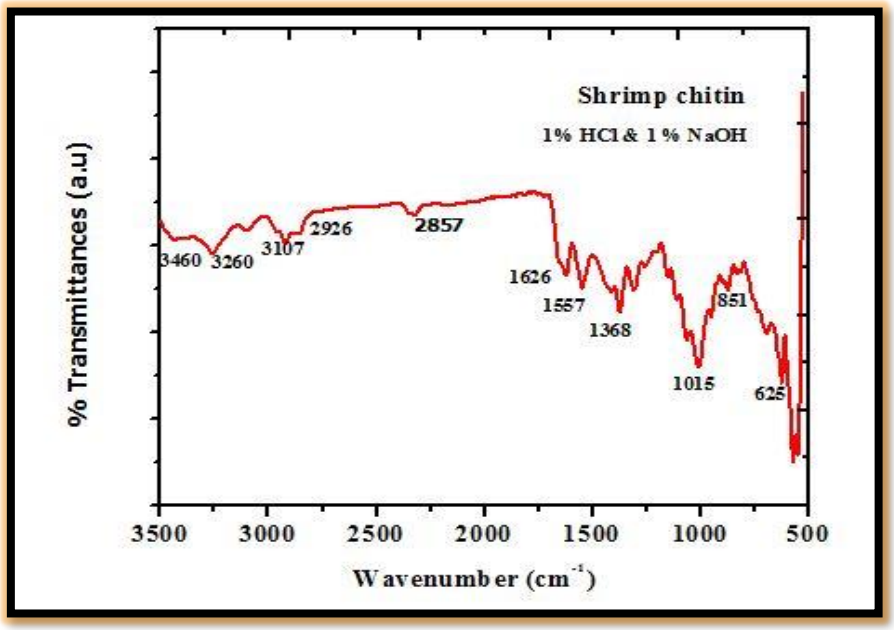

Figure 16: Shrimp chitin (1% HCl & 1% NaOH).

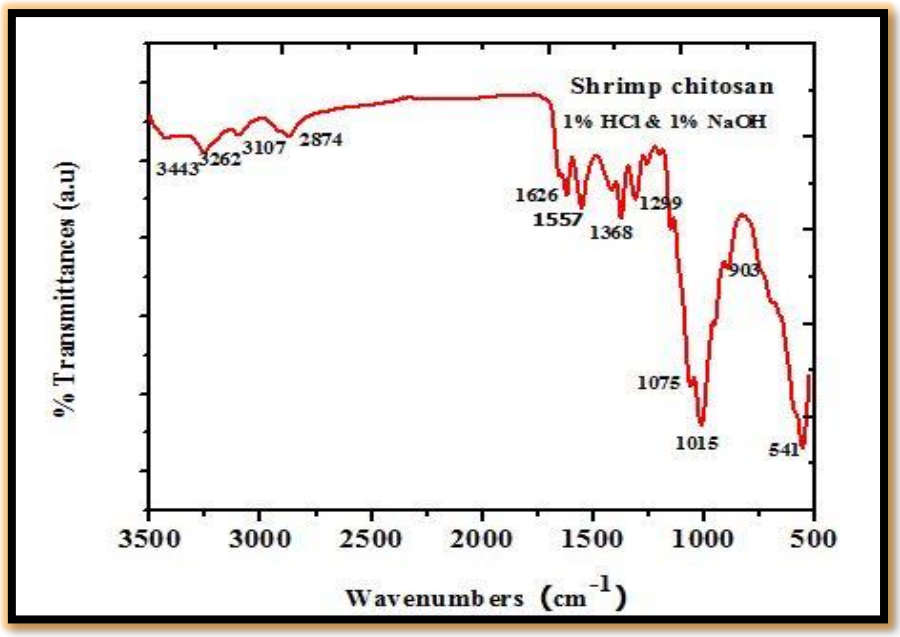

Figure 17: Shrimp chitosan (1% HCl & 1% NaOH).

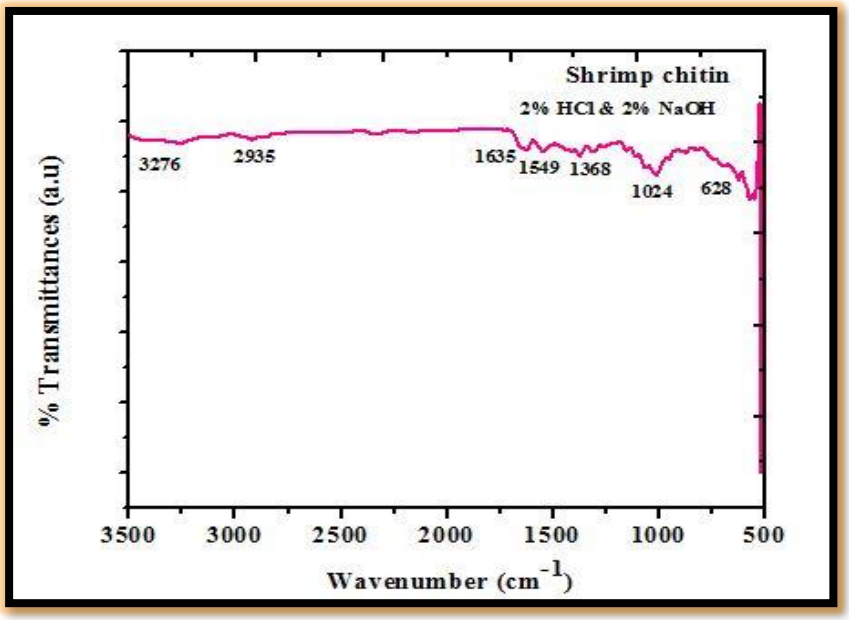

Figure 18: Shrimp chitin (2% HCl & 2% NaOH).

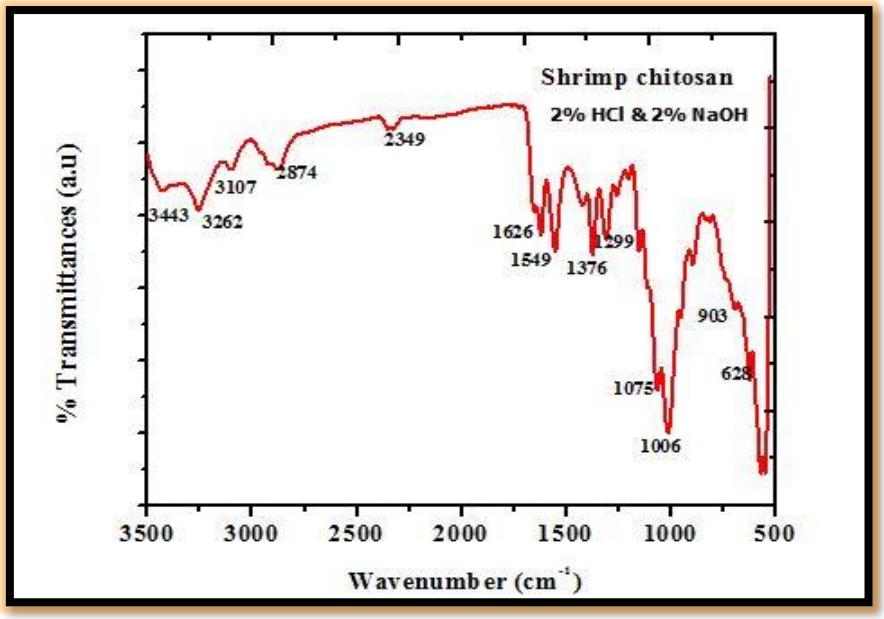

Figure 19: Shrimp chitosan (2% HCl & 2% NaOH).

4.4. FESEM

All the images are taken in the magnification range of 5000x. When the sample was studied based on its morphology with the help of a scanning electron microscope. Here in this figure, different magnifications of SEM images are shown and different areas of the samples are presented. From the above figures, the concluded structures of the biopolymer is porous and fibril shown in Fig [20 & 21] [38].

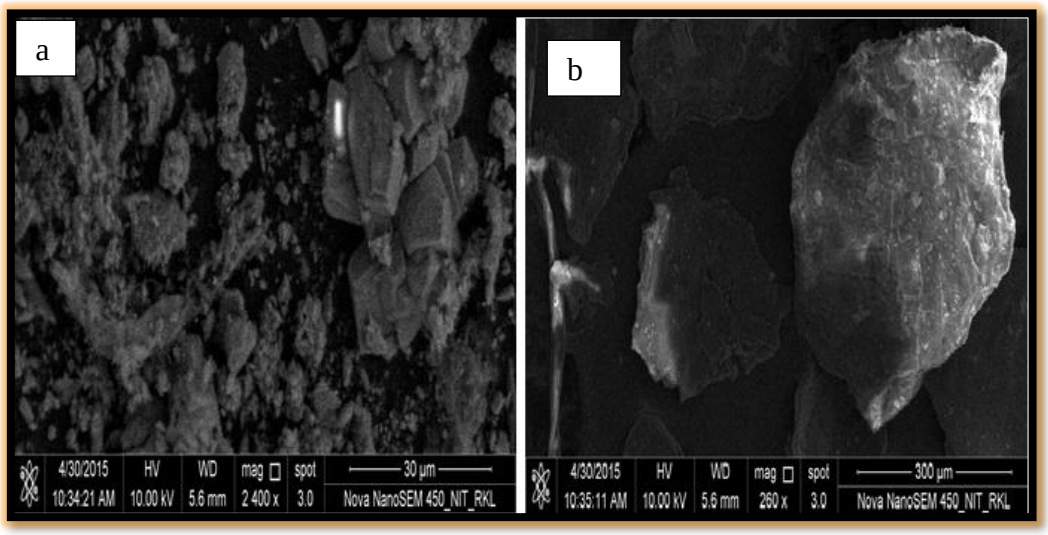

Figure 20: FESEM images of (a) 1% Crab Chitosan (b) 2% Crab Chitosan.

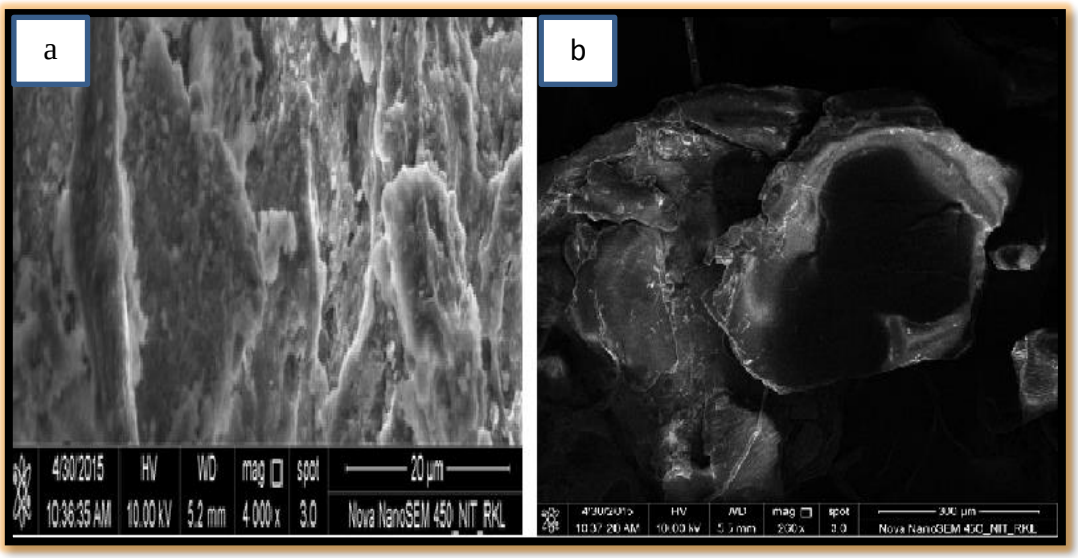

Figure 21: FESEM images of (a) 1% Shrimp chitosan (b) 2% Shrimp Chitosan.

4.5. Effects of various parameters

4.5.1. Effect of adsorbent dose

The effect of adsorbent Dosage on the adsorption of Cr^{+6} has been studied by taking 30 ml of 100 ppm Cr^{+6} solution in a conical flask and then different amounts of chitosan (i.e. From 0.1 cm to 0.4cm) were added in the different conical flask. Separate studies have been carried out for different samples of chitosan (i.e. chitosan from shrimp and chitosan from crab at certain pH (here pH= 4 i.e. acidic medium). After we kept the conical flask at 25°C in the shaker at 100 rpm for the time period of 30 minutes to obtain the concentration remaining in the solution after adsorption.

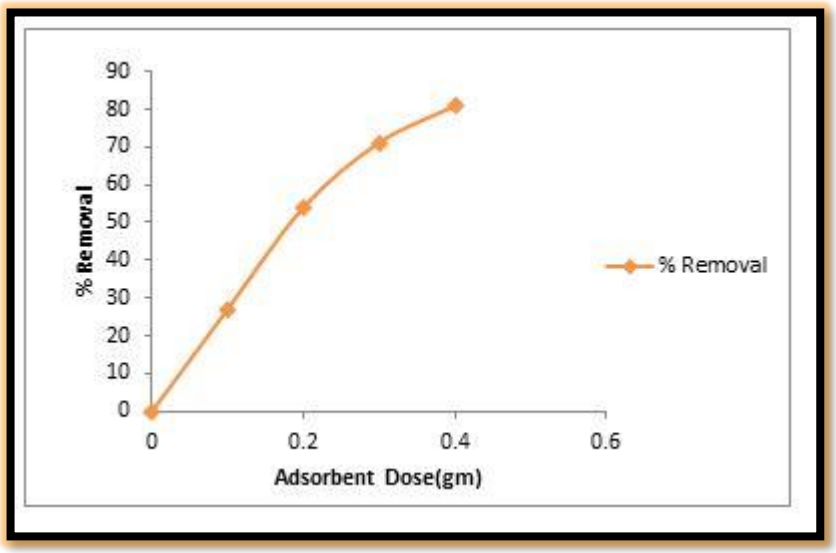

Figure 22: Graph between % Removal and Adsorbent dose for Crab chitosan.

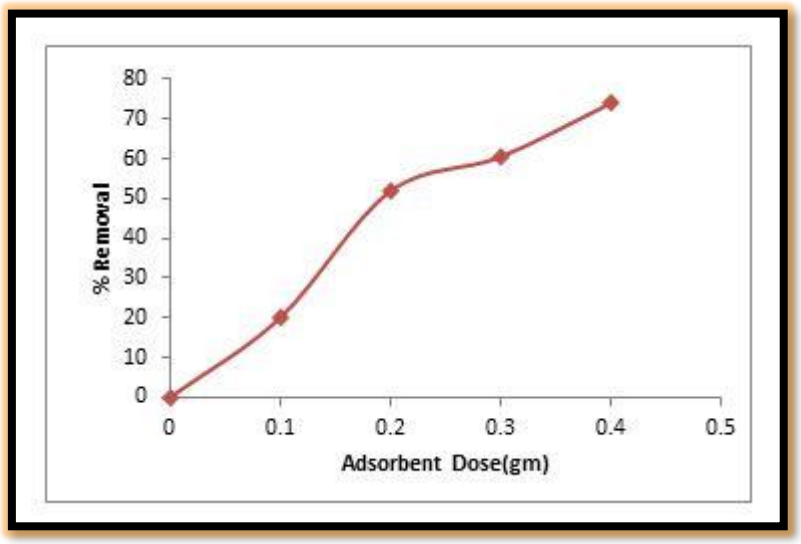

Figure 23: Graph between % Removal and Adsorbent for Shrimp chitosan.

From the graphs above obtained, it is found that as the dose of the adsorbent is increased keeping the time and speed of the shaker (rpm) constant, more percentage of Cr^{+6} is removed i.e.% of removal becomes more and more with the increased amount of adsorbent taken. For the case of crab chitosan, maximum 80.91 % removal of Cr^{+6} is obtained whereas maximum 73.89 % for the case of shrimp chitosan.

4.5.2. Effect of contact time

For the study of contact time on the adsorption of chitosan, 30 ml of 100 ppm Cr^{+6} solution is taken in a conical flask and 0.1gm of chitosan (both of crab and shrimp separately) is added in the flask at solution p^H. (Here pH= 4 i.e. acidic medium). The flask was kept at 25°C in the shaker at 100rpm shaking speed. Then the sample was pipetted out at the interval of 60 minutes initially and then at each interval of 60 minutes. pH After we kept the conical flask at 25°C in the shaker at 100 rpm for time period of 60 minutes to obtain the concentration remaining in the solution after adsorption.

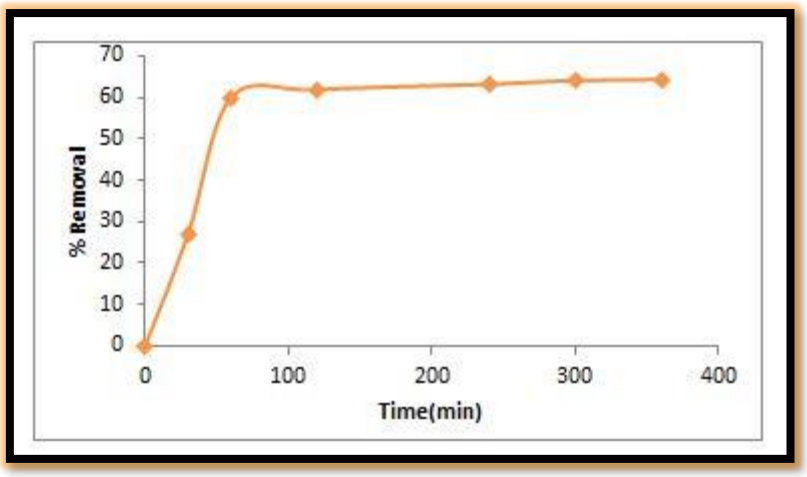

Figure 24: Graph between % Removal and Contact time for shrimp chitosan.

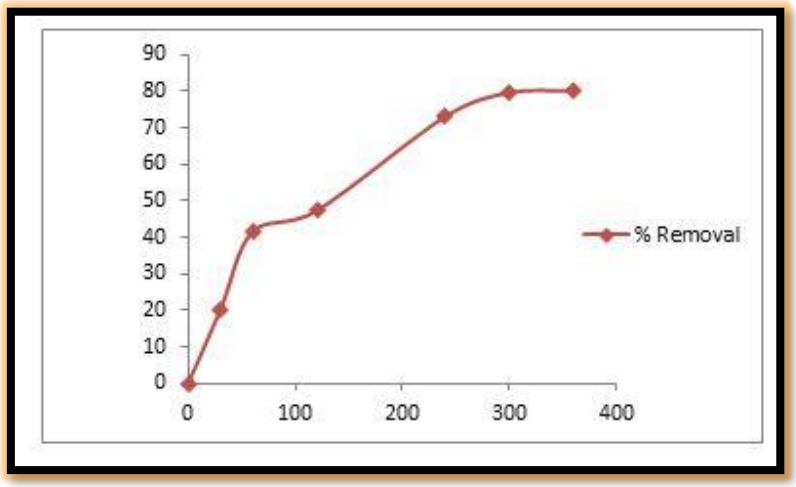

Figure 25: Graph between % Removal and Contact time for crab chitosan.

From the graphs obtained, it is found keeping the amount of adsorbent and speed of the shaker (rpm) that as the time progress ,the concentration of the heavy metal solution get decreasing, but after a certain time interval (i.e. 5-6 hours) it does not affect much. The percentage of removal of the heavy metal for the case of crab chitosan and shrimp chitosan are 80.11% and 64.29 % respectively.

CHAPTER- 5

CONCLUSIONS

5. CONCLUSIONS

- Here two types of samples of chitin and chitosan prepared from the corresponding raw materials of shrimp and crabs shells respectively and were found to be of good sources of chitosan.
- It is concluded that as the dose of the adsorbent is increased keeping the time and speed of the shaker (rpm) constant, more percentage of Cr^{+6} removed i.e.% of removal of Cr^{+6} becomes more and more with the increased amount of adsorbent taken.

- For the case of crab chitosan, maximum 80.91 % removal of Cr^{+6} was obtained whereas maximum 73.89 % for the case of shrimp chitosan studying the effect of adsorbent dose on the adsorption.

- It is concluded that keeping the amount of adsorbent and speed of the shaker (rpm) constant, with increased time of contact, the concentration of Cr^{+6} solution decreased. But after a lapse of about 5-6 hours, no further change in concentration was observed.

- The percentage of removal of Cr^{+6} for the case of crab chitosan and shrimp chitosan are 64.29 % and 80.11% respectively studying the effect of contact time on the adsorption process.

CHAPTER-6
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APPENDIX

Table 5: For Crab chitosan (30 min, 100rpm).

Adsorbent Dose(gm)	Concentration	% Removal
0	100	0
0.1	80.00312978	19.99687022
0.2	48.21011346	51.78988654
0.3	39.56089228	60.43910772
0.4	26.10232394	73.89767606

Table 6: For Shrimp chitosan (30 min,100 rpm).

Adsorbent Dose(gm)	Concentration	% Removal
0	100	0
0.1	72.9031647	27.0968353
0.2	45.96701874	54.03298126
0.3	28.8206506	71.1793494
0.4	19.08790061	80.91209939

Table 7: For Crab chitosan (0.1 gm,100 rpm).

Time(min)	Concentration	% Removal
0	100	0
30	80.00312978	19.99687022
60	58.50363713	41.49636287
120	52.7438261	47.2561739
240	26.95774142	73.04225858
300	20.35201755	79.64798245
360	19.88629026	80.11370974

Table 8: For Shrimp chitosan (0.1 gm,100 rpm).

Time(min)	Concentration	% Removal
0	100	0
30	72.9031647	27.09684
60	40.11216133	59.88784
120	38.13519648	61.8648
240	36.80454707	63.19545
300	35.94912959	64.05087
360	35.70200899	64.29799